Infections in PID Patients: Prevention of End-organ Damage, Need for Practice Guidelines and Screening

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Primary Immunodeficiency Diseases (PID's)

- Genetic defects of specific components of the immune system.
- More than 120 distinct syndromes have been described over the past 53 years.
- Recognized only when the person develops an infection, and not always even then.
- Permanent organ damage or death often occurs before the condition is diagnosed.

Immunodeficiency Diseases

- Acquired (most common)
  - $ Iatrogenic (chemotherapy,etc.)
  - $ Due to HIV (16,000 new adult and 1600 new childhood cases/day)
- Primary or genetically- determined (more than 120 distinct conditions) , involving:
  - $ B cells (most common)
  - $ T cells
  - $ Phagocytic cells
  - $ Complement

Clinical Presentation of Primary Immunodeficiency Diseases

Increased susceptibility to infection

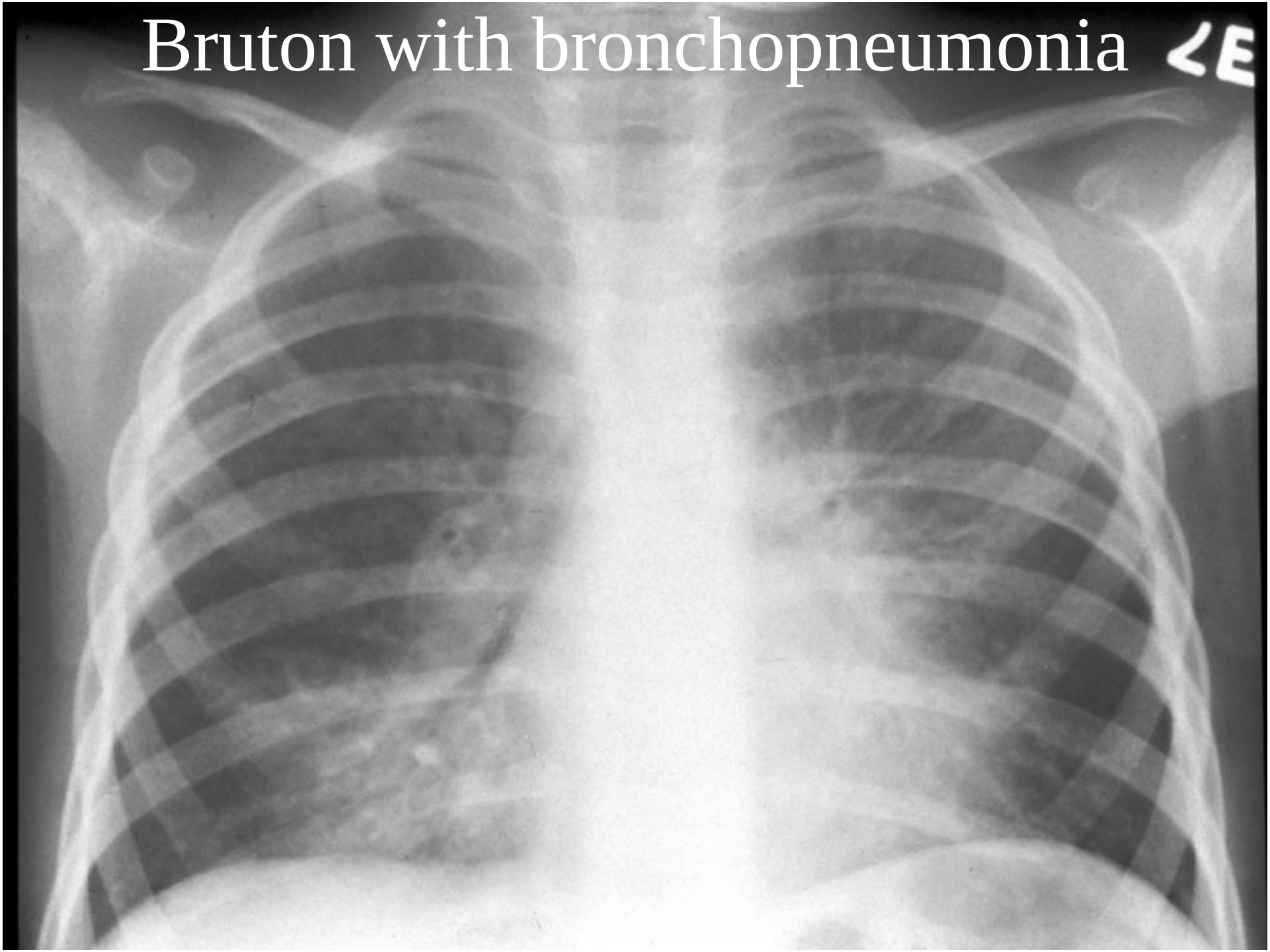
- Recurrent infections. Caveat: antibiotics may mask.
- Unusually severe.
- Unusually persistent/complicated.
- Organisms of low virulence.

Outwardly, these patients appear normal. With no screening, need high index of suspicion

Infectious Agents in Immune Disorders

Pathogens:	Defect in:				
	T-cells	B-cells	Granulocytes	Monocytes	Complement
Bacteria		Pneumo, Staph H. flu, Strept Mycoplasma	Staph Pseudomonas Serratia	Mycobacteria Salmonella	Neisseria Pneumo
Viruses	Herpes family, RSV, etc.	Enteroviral encephalitis			
Fungi, Parasite	Candida Pneumocystis	Giardia	Candida Aspergillus		
Special features	Severe, persistent	Respiratory	Abscesses, cellulitis		Autoimmunity

Bruton with bronchopneumonia

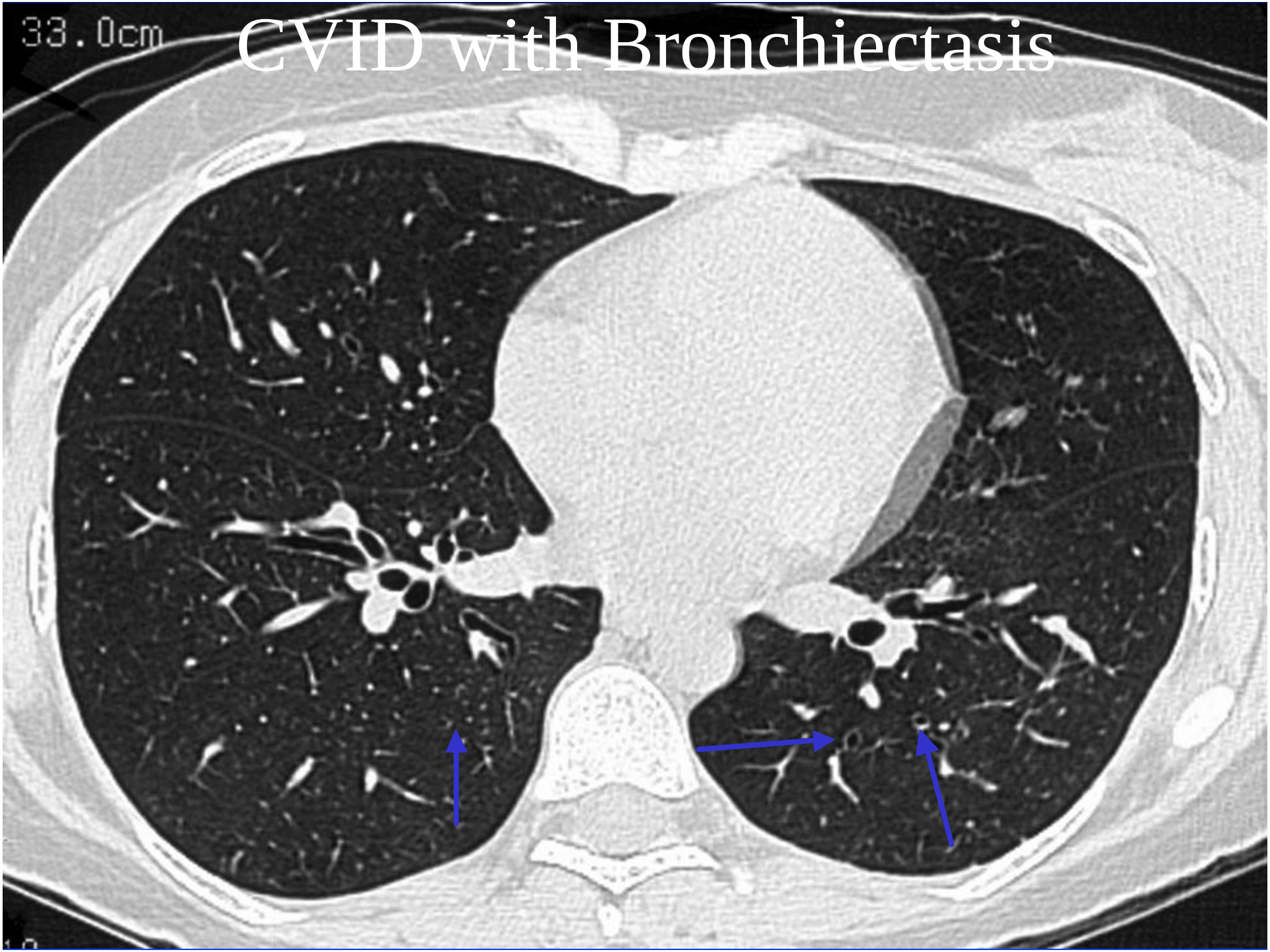


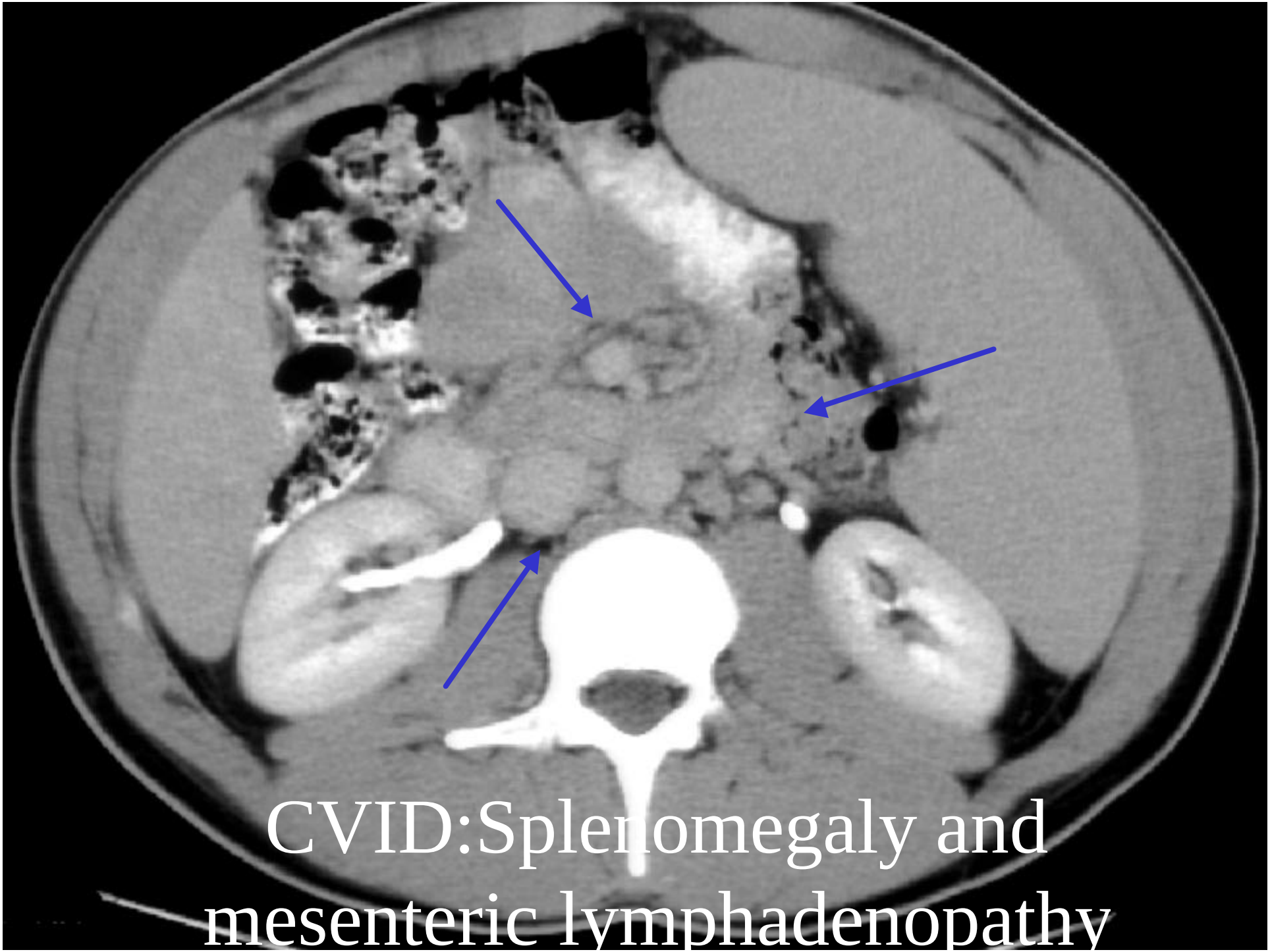
Enteroviral Meningoencephalitis in Bruton's Disease and in CVID

- Due to infections with many different enteroviruses*. Echovirus 11 is the most common.
- Live polio vaccine also causes this.
- Results in permanent CNS damage or death.
- Incidence much less now than before IVIG was developed.
- However, this condition can still develop if there is a delay in diagnosis.

*Halliday, E et al J Infect 46:1-8, 2003

CVID with Bronchiectasis





CVID:Splenomegaly and mesenteric lymphadenopathy

Changing Concepts

- Recurrent pneumococcal infections, consider the following diagnoses*:
  - Most B and T cell defects
  - Deficiencies of the early complement components
  - Congenital asplenia
  - NEMO (ectodermal dysplasia with immunodeficiency)
  - IRAK4 deficiency

*Picard, C. et al. Current Opin Allerg Clin Immunol 3: 451-459, 2003

Changing Concepts (cont'd)

- Infections with mycobacteria and/or salmonella raise the possibility of an interferon gamma receptor deficiency, or an IL-12 or IL-12 receptor defect or STAT-1 deficiency.
- Infections with Cryptosporidium and/or *Pneumocystis carinii* lead to suspicion of X-linked Hyper IgM.

Changing Concepts (cont'd)

- Previously Serratia, Staphylococcus and aspergillus species were pathognomonic of chronic granulomatous disease (CGD).
- Fungal infections in are now becoming more diverse in CGD, such as those with Trichosporon pullulans* and Penicillium.

*Moylett EH et al: JACI 111:1370-1374, 2003

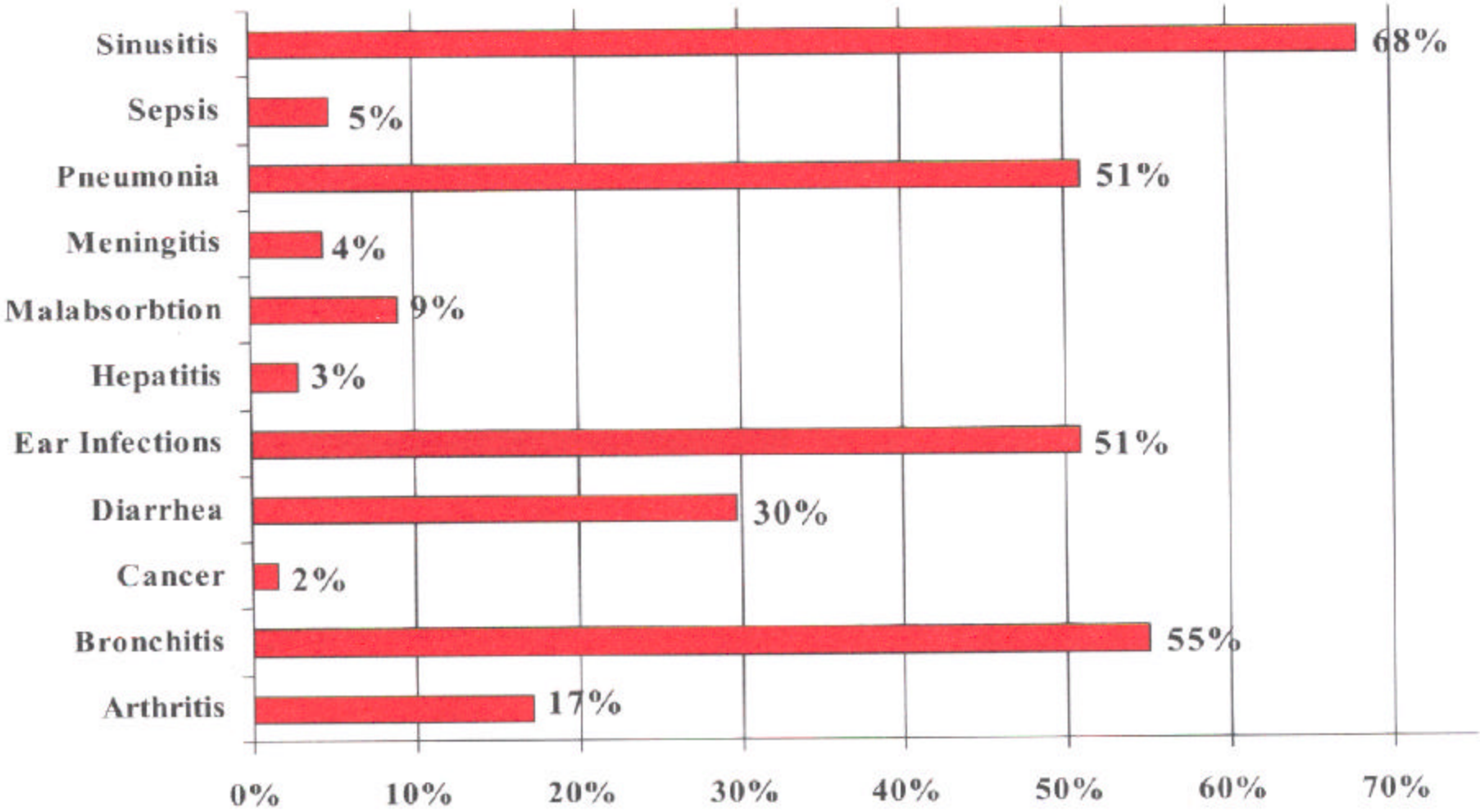
New Threats for PID

- West Nile virus.
- Smallpox or exposure to family members immunized against it.
- Anthrax.

Incidence and Prevalence of PID

- Various estimates but basically unknown.
- No newborn screening.
- Use of live vaccines at birth (e.g. BCG) or during infancy (Varivax) makes death almost certain for those with genetic defects in T cell function.
- Widespread overuse of antibiotics masks the textbook clinical presentation of primary immunodeficiency. IDF survey: average time to diagnosis was 9.7 years from first infection.

Conditions before Diagnosis



Source: IDF Patient Survey N=2,807

Current Status

- Currently no screening for any genetic defect of the immune system at birth or at any time during life, anywhere in the world.
- A major problem in those countries where all infants are immunized with live BCG vaccine on day 1 of life to prevent tuberculosis.
- Infants with defects in cellular immunity usually die from generalized infections with the vaccine organisms.

Paradox

- Screening methods are available and could easily be implemented if screening for these defects were accepted as the standard of care.
- Obstacle: these defects are generally considered to be so rare that screening is not cost-effective.

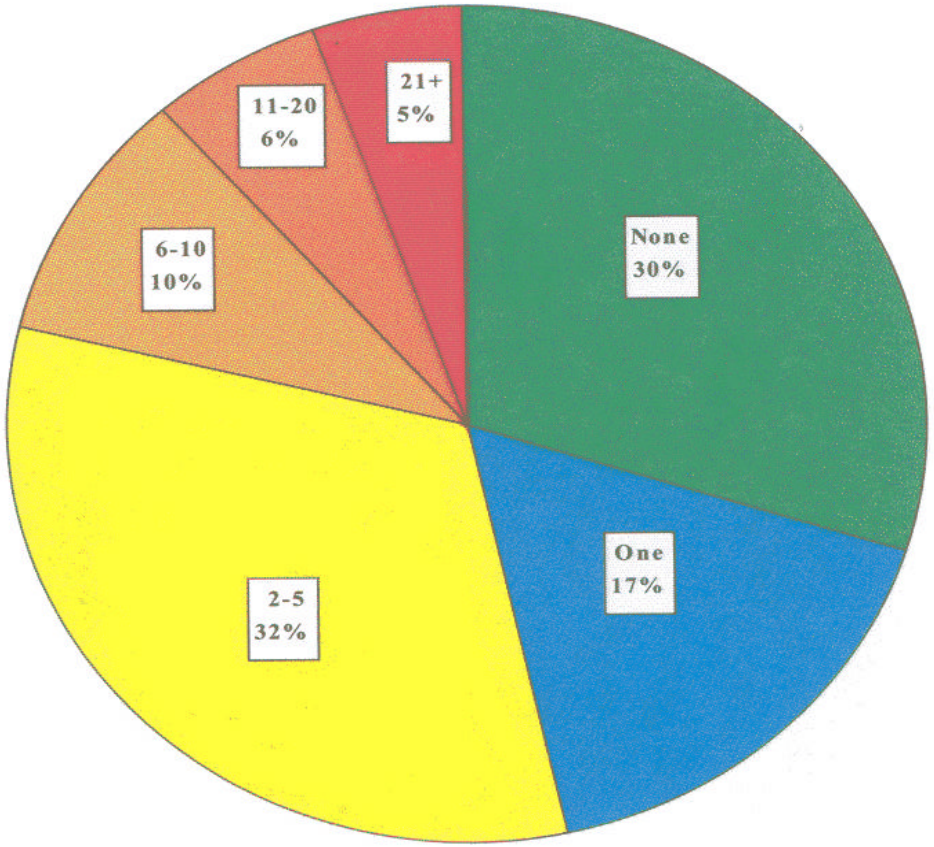
Conundrum

- Population surveys suggest that PID's affect an estimated 50,000 persons in the U.S. and that they are at least as common as hemophilia (<15,000), cystic fibrosis (30,000), Huntington's Disease (30,000) and phenylketonurea (<18,000).
- However, true incidences will not be known until there is population screening.

The Question of Cost

- Half of all persons with PID's are not diagnosed until they are adolescents or older.
- The cost of late diagnosis is a heavy burden of disease on the patient and early demise.
- The majority of patients report two or more hospitalizations before diagnosis. The cost of hospitalization of these patients far exceeds what it would cost to screen for the defect and to implement therapeutic or preventive measures.

Times Hospitalized before Diagnosis



Source: IDF Patient Survey N=2,708

Screening for Primary Immunodeficiencies

- Selective IgA deficiency
  - most common primary immunodeficiency—as frequent as 1:333 persons in U.S. Thus, there could be as many as 1 million people in the U.S. with this or other PIDs.
  - could be screened for by measuring serum IgA on a heel stick done when the routine hemoglobin is checked at 10-12 months of age.
  - Same study could be done when pre-school immunizations are given at age 5-6 years and during pre-college physical examinations.

Screening for Other Primary Immunodeficiencies

- The agammaglobulinemias
  - If the IgA level is found to be low in screening, levels of the other immunoglobulins should be measured immediately to rule in or out the agammaglobulinemias, so that IVIG can be started before organ damage occurs.
- Genomic and proteomic methods currently exist that would make screening for all of these defects at birth eventually possible.

Causes of Death in 32 SCIDs After Marrow Transplantation

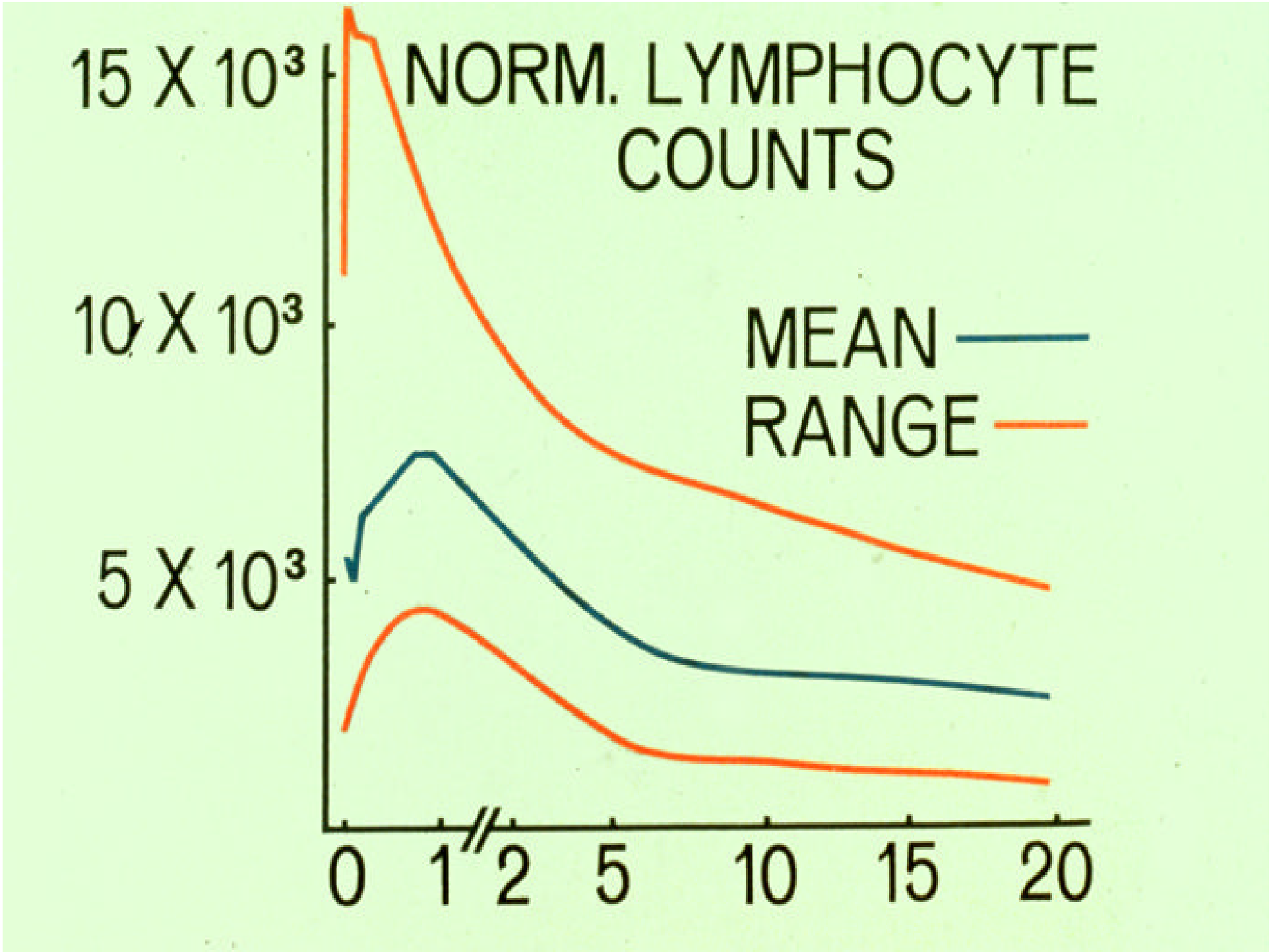
CMV	8
Adenovirus	7
EBV /Lymphoma	4
Enterovirus, Rotovirus	3
Parainfluenza 3, Varicella	2 ea
Herpes simplex/RSV	1 ea
Candida sepsis	2
Pulmonary disease	2
Mitochondrial defect	1
Nephrotic syndrome/chemo	1
VOD	1

What is the Prevalence and Incidence of SCID?

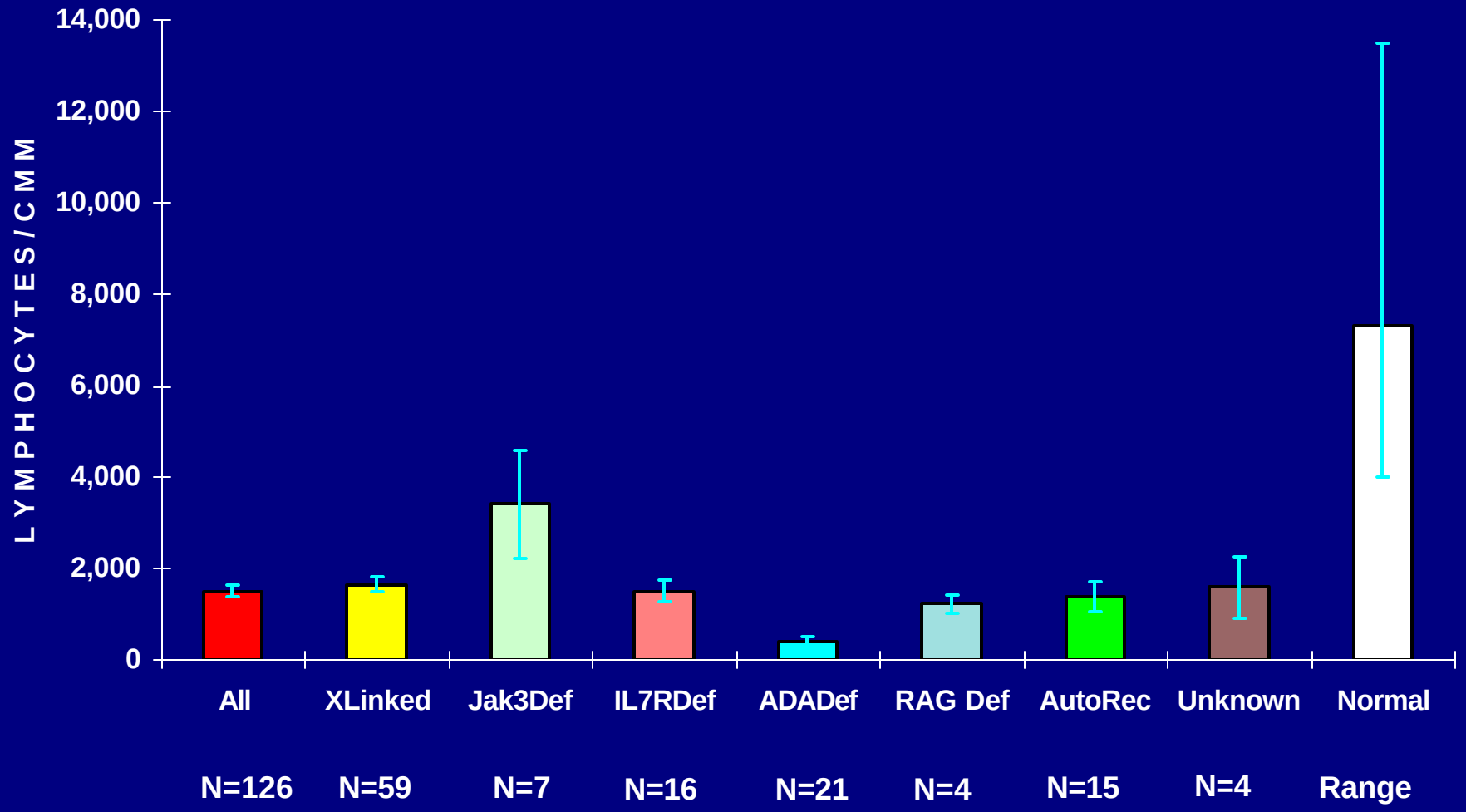
- Prevalence must be very low, because it is uniformly fatal in infancy unless corrected by immune reconstitution.
- What is the incidence? Unknown. Estimated at 1:100, 000 live births. I suspect is much higher—possibly as high as phenylketonurea, e.g. 1:16,000.

Is there an Existing Test that Could be Used to Screen for SCID?

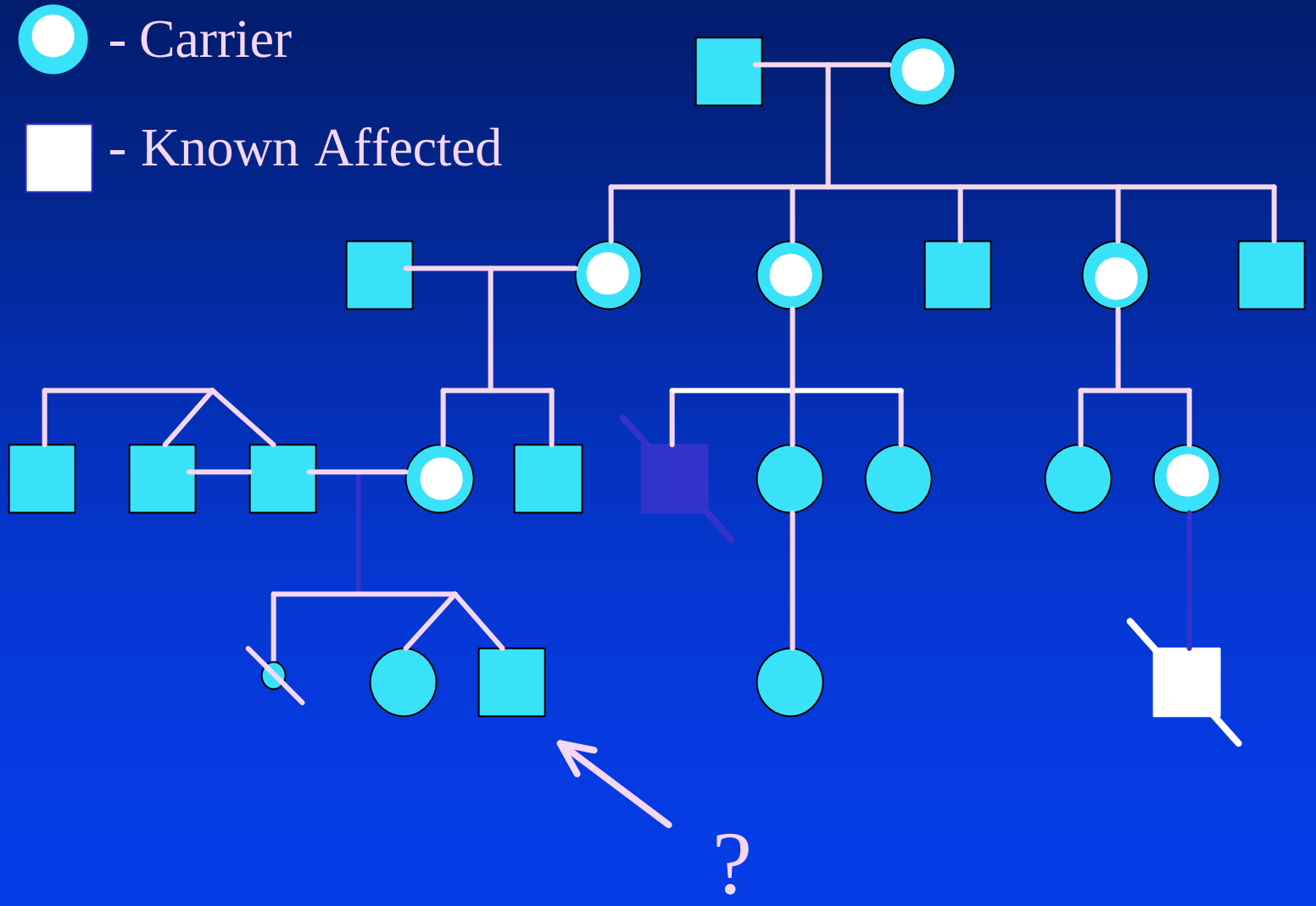
- Yes. What is the test? Absolute lymphocyte count (ALC). This requires that a white blood cell count and a manual differential be done on the cord blood.
- Is it a screening or a diagnostic test? Screening.
- Is management of the process centralized? No. Not usually performed because of cost and other considerations.


15 X 10³
10 X 10³
5 X 10³
NORM. LYMPHOCYTE COUNTS
MEAN
RANGE
0
1
2
5
10
15
20

Mean Absolute Lymphocyte Counts in 126 SCIDs Pre-Transplantation



XSCID Pedigree



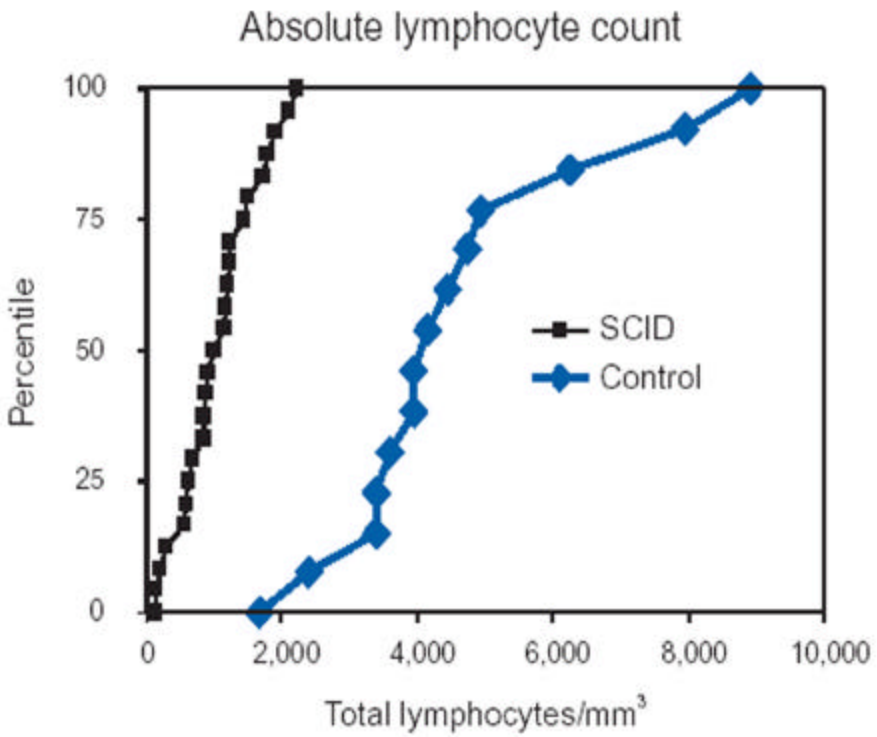
Cord Blood White Cells

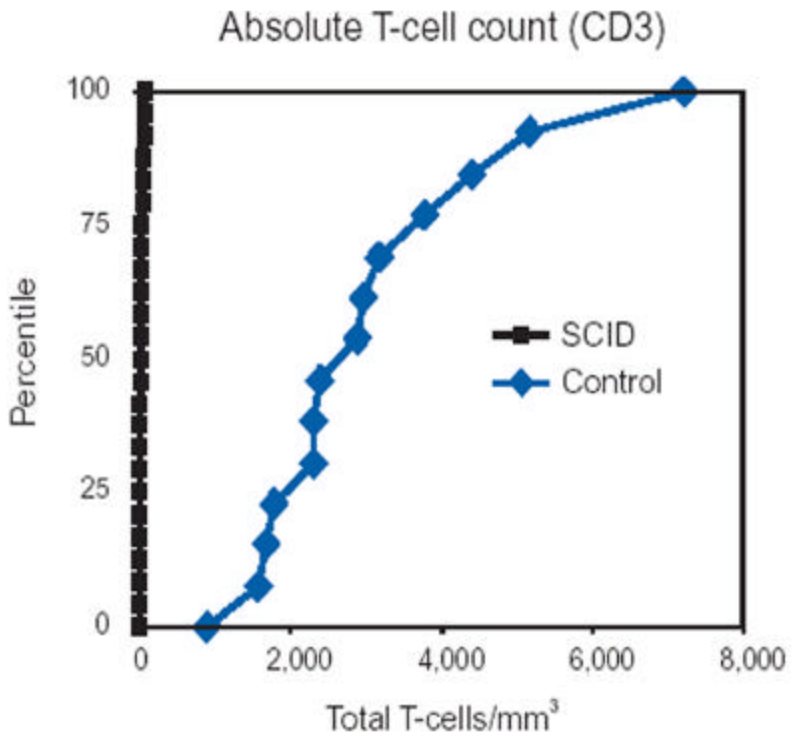
Parameter	Boy	Girl	Normal
WBC	6,700	17,700	18,100 (9,000 -30,000)
Absolute lymphocyte count	2,211	5,133	5,500 (2,000-11,000)

Flow Cytometry (cells/mm^3)

Subtype	Boy	Girl	Normal
CD3+ T Cells	23	1687	4072 (1481-8145)
CD4+ T Cells	3	1266	2475 (900-3424)
CD8+ T Cells	7	590	1581 (172-2309)
NK Cells	11	502	557 (203-1114)
B Cells	1198	886	527 (192-1055)

Absolute Lymphocyte Count Distributions in SCID: 25 SCID and 14 Healthy Newborns at Birth





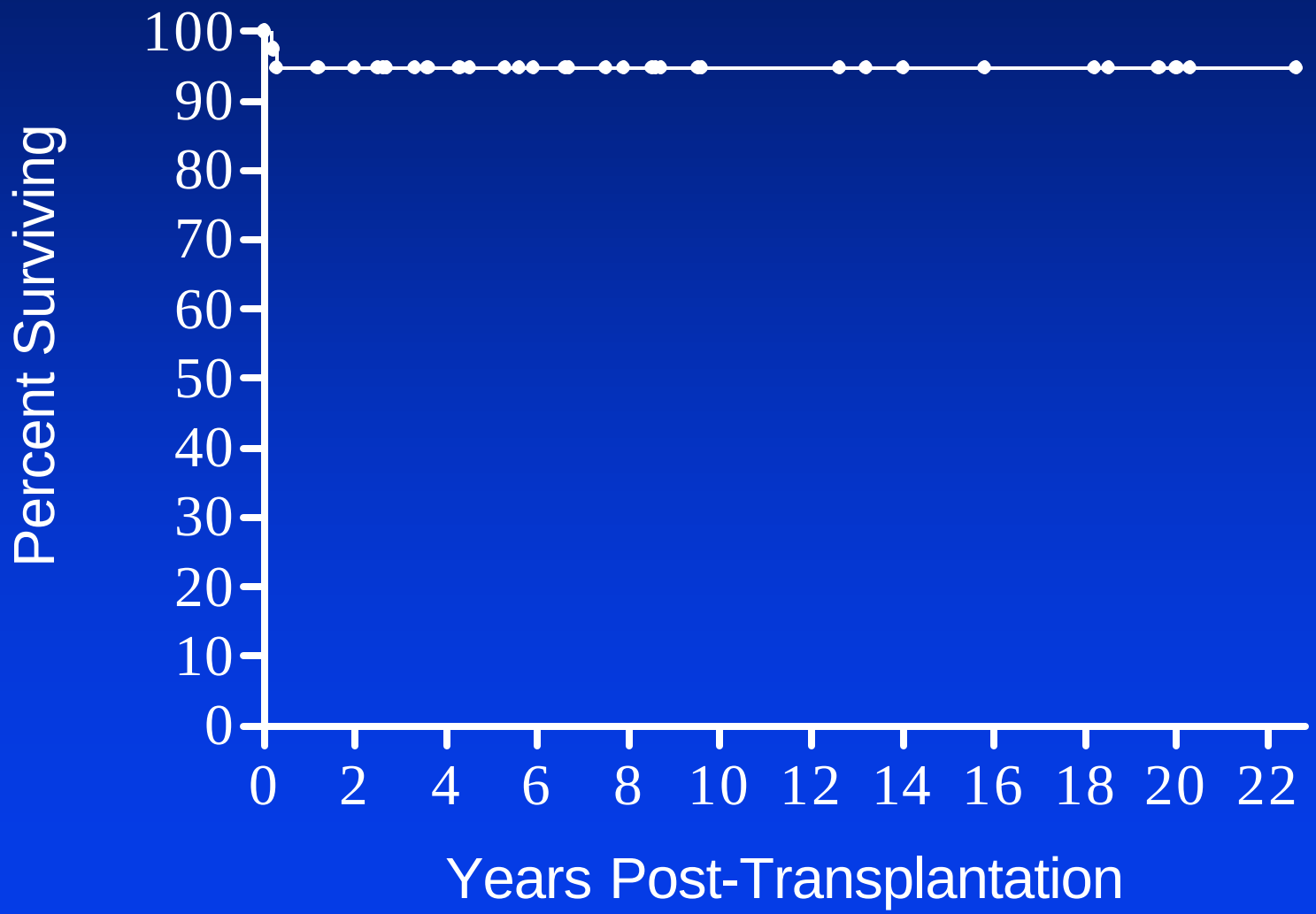
Follow-up Testing

- What diagnostic tests will follow the screening test?
  - Repeat white cell count and manual differential by peripheral heel stick.
  - If lymphopenia is confirmed (i.e, <2,500/mm^3), then do flow cytometry to count T, B and NK cells.
- If there is an absence of T cells, the diagnosis should be confirmed by T cell functional studies, but the infant should be kept in protective isolation and plans for intervention initiated immediately.

Intervention

- For positive diagnostic tests, what therapeutic intervention will follow?
  - Non-chemoablative, related donor bone marrow transplantation with no GVHD prophylaxis, soon after birth.
- If infant is healthy, the procedure can be done as an outpatient so he or she will not be exposed to nosocomial infections.
- Cost of doing this as an outpatient is less than $50,000. Cost of doing this if diagnosis is made later is primarily for ICU treatment of serious infections, e.g. hundreds of thousands of dollars.

Kaplan Meier Plot of 39 SCIDs Transplanted in the First 3.5 Months of Life



Test Performance

What is:

- The detection rate? >95%.
- The false negative rate? Rare SCID patients with large numbers of transplacentally-transferred T cells may have a normal ALC. Omenn's and ZAP70 deficient patients would have high ALC.
- The false positive rate? Low. Other cellular immunodeficiencies that could have lymphopenia at birth include: HIV infection, PNP deficiency, MHC Class I or II deficiency, complete DiGeorge.
- The odds of being affected with a T cell defect, given a positive result? > 95%.

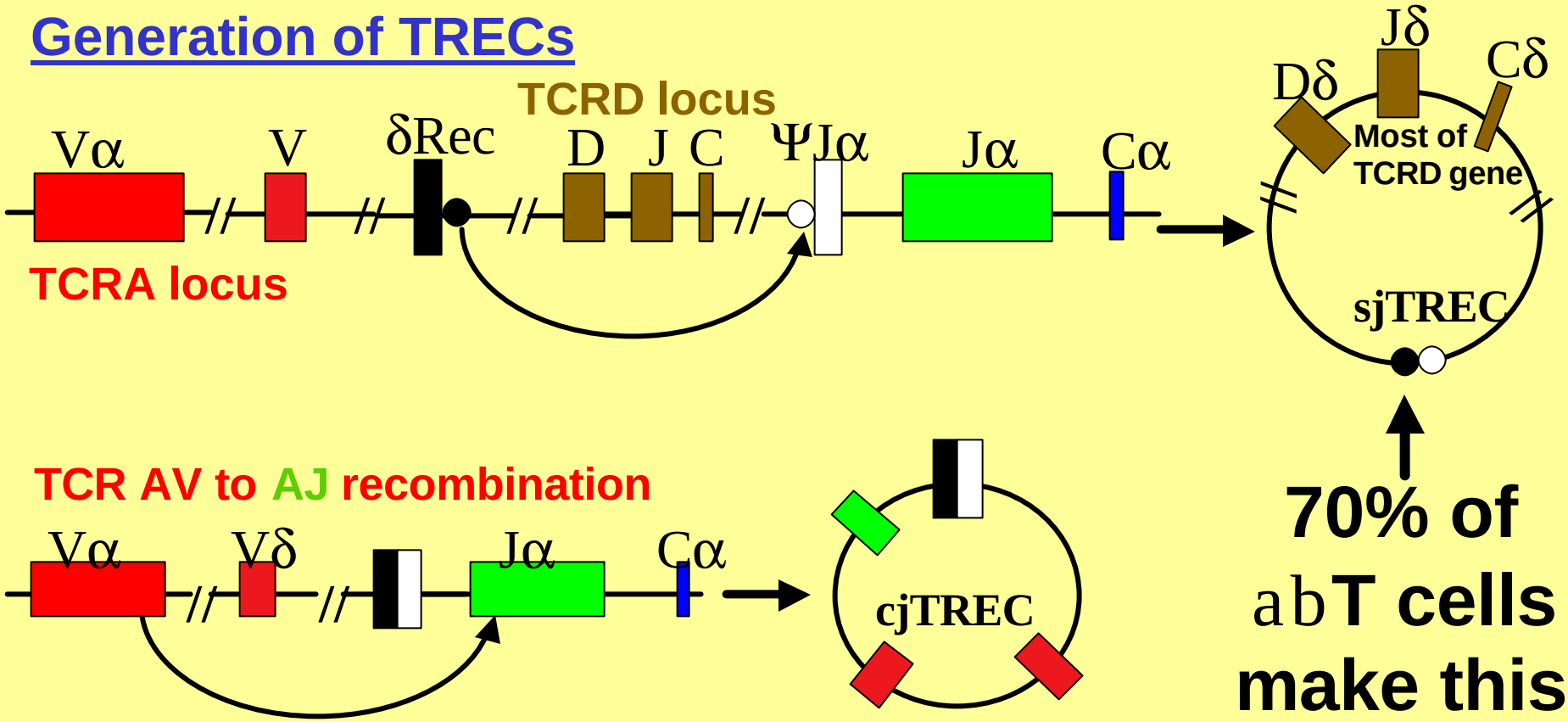
Practical Aspects

- What are the practical problems in implementation?
  - Can’t be done on the Guthrie spot.
  - Has to be done on anticoagulated blood within 24h.
  - Nucleated red cells interfere with machine differentials.
  - Automated blood counts and manual differentials are expensive when compared to other newborn screening tests.
- Are special facilities required? No.
- Unlike the Guthrie Spot results, the ALC results will be known immediately!

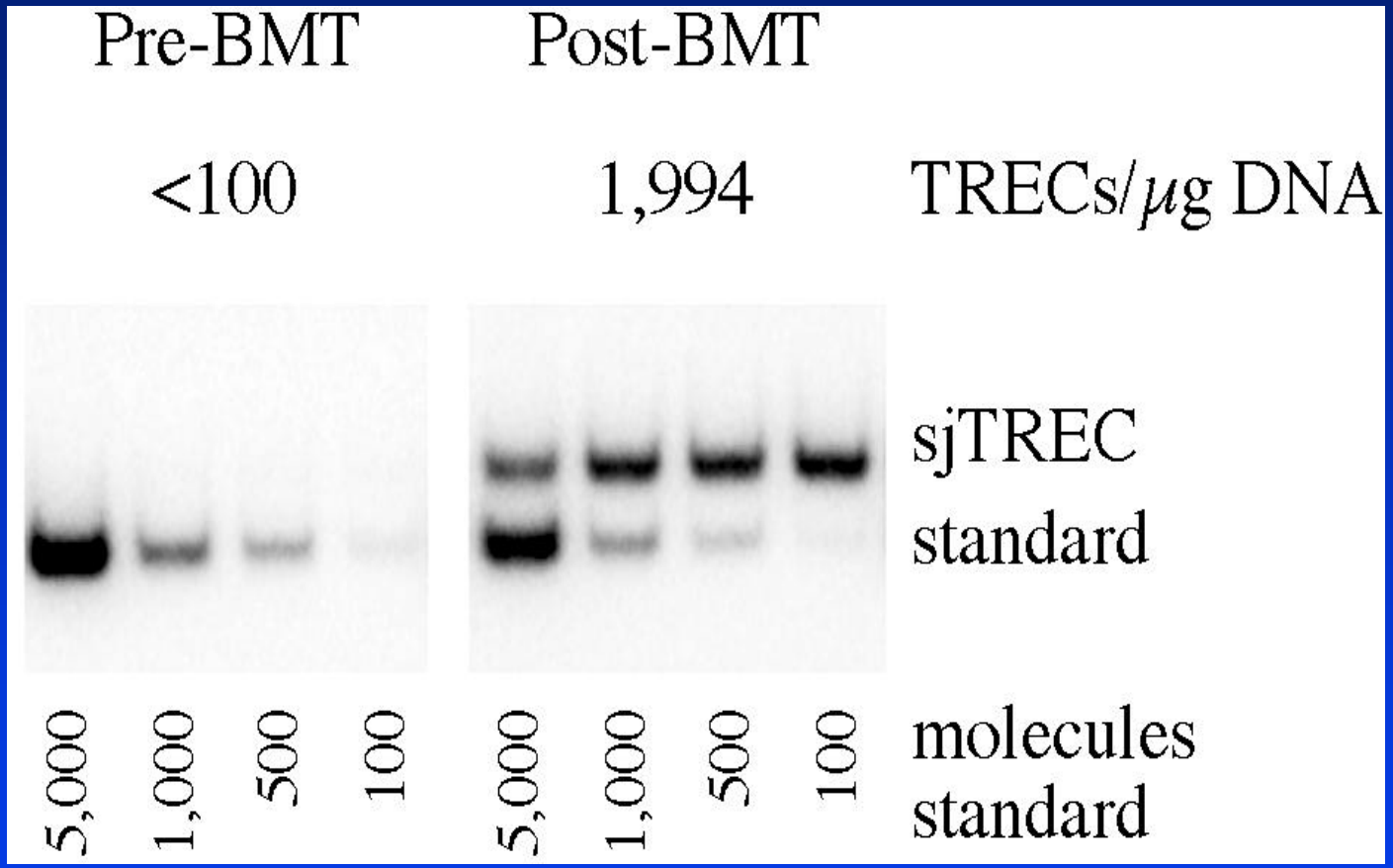
Output of New T cells from the Thymus

- Thymus produces new naïve T cells with antigen specificity determined by T cell receptor (TCR) DNA recombination.
- TCR recombination makes episomal circles (TRECs) as a byproduct
- TRECs are stable and can be detected by PCR in newly formed T cells, but are diluted out as T cells undergo many divisions
- Newborns have the most TRECs; TRECs decrease as thymus involutes

Generation of TRECs



sjTREC Assay

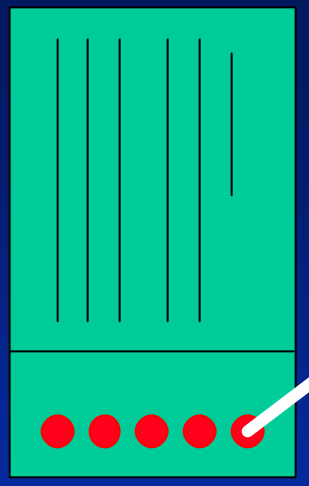


T-Cell Counts and Thymic Output in SCID Infants Before Transplantation

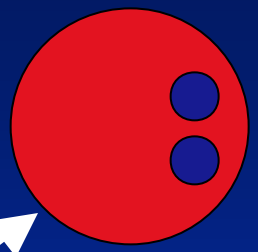
Patient	Type	TRECs	CD3 Count
1	Autosomal Recessive	0	100
2	Jak3-/-	3	8268‡
3	X-linked	0	17
4	X-linked	8	21
5	X-linked	226	651‡
6	X-linked	100	365‡
7	X-linked	9	15
8	X-linked	6	42
9	X-linked	1	570‡
10	X-linked	5	904‡
11	X-linked	17	23
Mean ± SD		34 ± 70	998 ± 2,431

‡ Documented transplacental-transfer of maternal T cells into the fetal circulation.

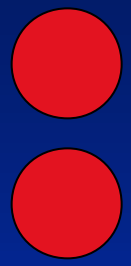
TREC Assay Procedure



Guthrie Card



50 ul blood

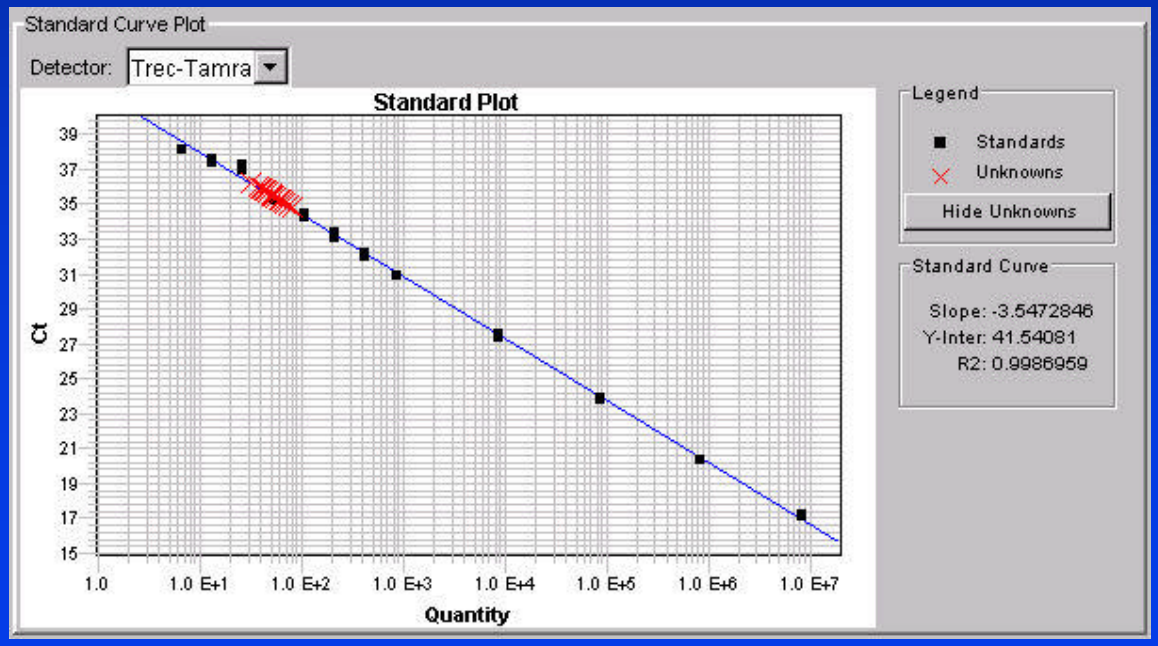


3ul

3ul

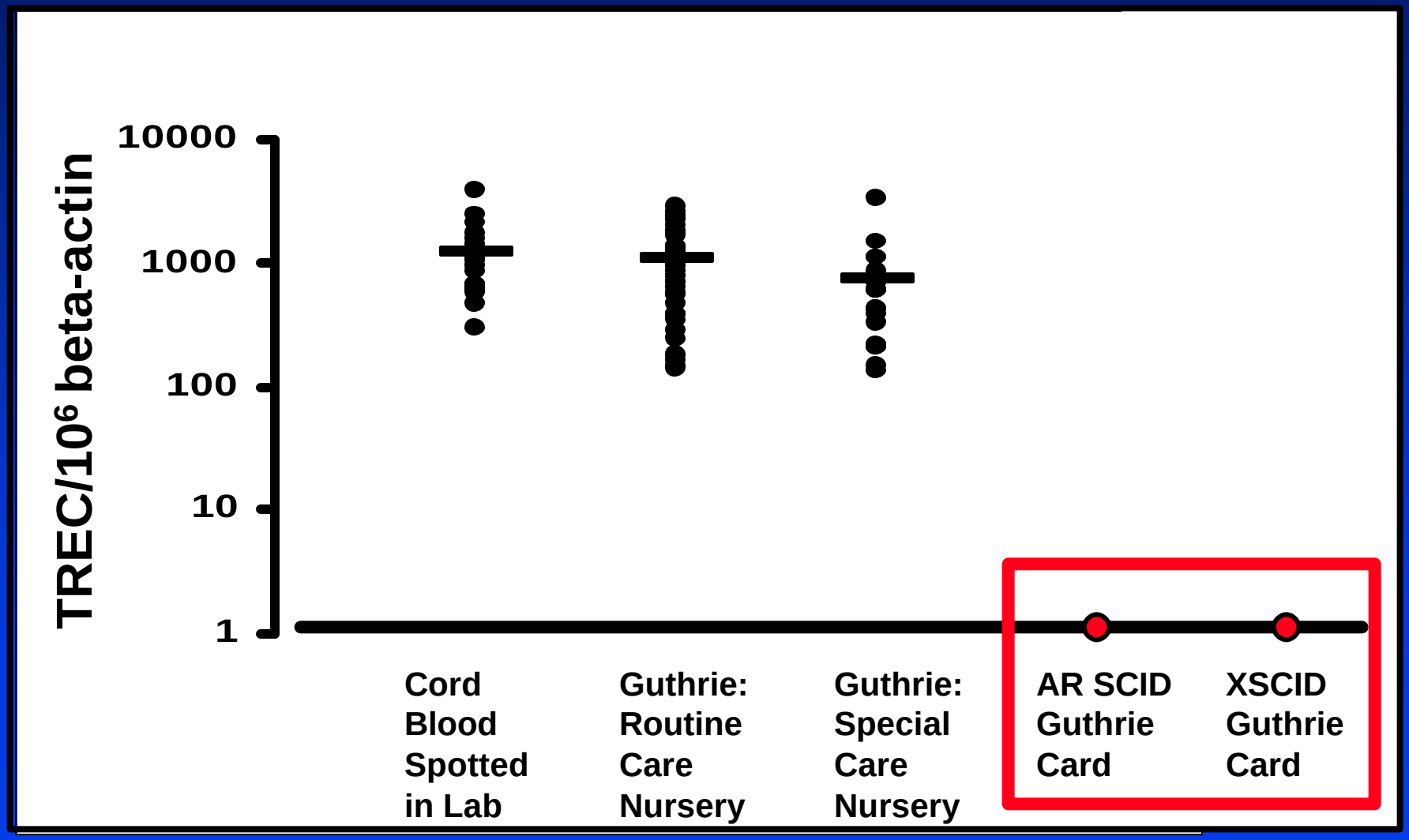
2 holes punches from blood spot

DNA Extraction



Quantitation of TRECs by Real-time PCR

TRECs in newborn samples



Conclusions

- The TREC assay is a promising tool for large-scale newborn screening for SCID.
- Future studies will
  - Scale up throughput for pilot testing.
  - Investigate causes of false positives.
  - Establish the true incidence of T-cell immunodeficiencies.
  - Define the range of genotypes with low TRECs.

Clinical Care Guidelines

- In the absence of screening, these could have a significant impact on the time of diagnosis of PID.
- As defined by the IOM's 1990 Report practice guidelines are "*systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances*".
- May also be used for quality improvement and payment policymaking.

Clinical Care Guidelines (cont'd)

- These are being developed for PID by the Immune Deficiency Foundation and will soon be posted on the IDF website.
- They are written so that patients and their families can understand them and call their physician's attention to the most appropriate testing and therapy currently available.
- There is no stronger advocate for the patient than the patient and his or her family.